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ABSTRACT

This column highlights recently published articles that are of interest to the readership of this publication. We encourage ABRF members to forward information on articles they feel are important and useful to Clive Slaughter, AU-UGA Medical Partnership, 1425 Prince Avenue, Athens GA 30606. Tel; (706) 713-2216: Fax; (706) 713-2221: Email; cslaught@uga.edu or to any member of the editorial board. Article summaries reflect the reviewer's opinions and not necessarily those of the Association.

NUCLEIC ACID SEQUENCING

Hagemann-Jensen M, Ziegenhain C, Sandberg R. Scalable single-cell RNA sequencing from full transcripts with Smart-seq3xpress. ***Nature Biotechnology*** **40;2022:1452-1457.**

Hahaut V, Pavlinic D, Carbone W, Schuierer S, Balmer P, Quinodoz M, Renner M, Roma G, Cowan C S, Picelli S. Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq. ***Nature Biotechnology*** **40;2022:1447-1451.**

Improvements to methodology for single-cell RNA sequencing are described by two groups. Their aim is to narrow the gap in capability between droplet methods with combinatorial indexing, which provide high cell throughput but limited depth of transcriptome analysis, and plate-based methods, which provide lower cell throughput but deeper transcriptome analysis. Hagermann-Jensen *et al*. represent the group that formulated the plate-based Smart-seq3 method, which currently provides the highest information content per profiled cell. They miniaturize and streamline their original protocol. Reaction volumes are reduced to the nanoliter scale, avoiding evaporation by covering with an inert, hydrophobic substance, and the protocol is simplified substantially to increase cell throughput and reduce reagent consumption. Hahaut *et al*. achieve similarly significant streamlining and protocol simplification and accelerated throughput.

Battaglia S, Dong K, Wu J, Chen Z, Najm F J, Zhang Y, Moore M M, Hecht V, Shoresh N, Bernstein B E. Long-range phasing of dynamic, tissue-specific and allele-specific regulatory elements. ***Nature Genetics*** **54;2022:1504-1513.**

Prevailing methods for mapping chromatin accessibility and modification typically rely upon short-read sequencing lacking capability for detailed analysis of repetitive regions and chromosome phasing of epigenetic features. Battaglia *et al.* here contribute to methodology for combining Oxford Technologies long-read nanopore sequencing and exogenous, *in situ* marking of accessible DNA with a CpG methyltransferase to profile chromatin accessibility. The authors target regions of interest by directed cleavage of high-molecular-weight genomic DNA using CRISPR-Cas9. They sequence the resulting fragments, up to 116 kb in length. Using phase information from the single-molecule data, they study imprinting mechanisms at the extensively studied insulin-like growth factor 2/H19 locus, charting the role of CTCF insulator binding sites in imprinting.

Tanić M, Moghul I, Rodney S, Dhami P, Vaikkinen H, Ambrose J, Barrett J, Feber A, Beck S. Comparison and imputation-aided integration of five commercial platforms for targeted DNA methylome analysis. ***Nature Biotechnology*** **40;2022:1478-1487.**

Bisulfite sequencing remains the standard method for study of DNA methylation, an epigenetic mark of key importance in determining gene activity. While high-throughput, whole-genome bisulfite sequencing provides the most comprehensive information about DNA methylation, smaller scale bisulfite sequencing studies that are targeted toward particular regions have also proven highly informative, whether they're designed to analyze particular regions isolated by specific probe hybridization capture, or designed to analyze non-specifically enriched regions of high CpG density (reduced-representation bisulfite sequencing – RRBS). To help in the choice of the most suitable experimental platform for any particular targeted investigation of DNA methylation, a benchmark study here compares the performance of 5 standardized, commercially available kits: for probe hybridization capture, Agilent SureSelect Methyl-Seq, Roche NimbleGen SeqCap EpiGiant, and Illumina TruSeq Methyl Capture EPIC; and for RRBS, Diagenode Premium RRBS, and NuGen Ovation RRBS Methyl-Seq. The study uses a panel of 11 different samples. The results for 2 samples are also compared with whole-genome sequencing from Illumina and Oxford Nanopore platforms as gold-standard data-sets. Of particular interest in this work, imputation is used to compare the performance of different platforms that inevitably provide information about CpG sites that are only partially overlapping.

Karthikeyan S, Levy J I, De Hoff P, Humphrey G, Birmingham A, Jepsen K, Farmer S, Tubb H M, Valles T, Tribelhorn C E, Tsai R, Aigner S, Sathe S, Moshiri N, Henson B, Mark A M, Hakim A, Baer N A, Barber T, Belda-Ferre P, Chacón M, Cheung W, Cresini E S, Eisner E R, Lastrella A L, Lawrence E S, Marotz C A, Ngo T T, Ostrander T, Plascencia A, Salido R A, Seaver P, Smoot E W, Mcdonald D, Neuhard R M, Scioscia A L, Satterlund A M, Simmons E H, Abelman D B, Brenner D, Bruner J C, Buckley A, Ellison M, Gattas J, Gonias S L, Hale M, Hawkins F, Ikeda L, Jhaveri H, Johnson T, Kellen V, Kremer B, Matthews G, Mclawhon R W, Ouillet P, Park D, Pradenas A, Reed S, Riggs L, Sanders A, Sollenberger B, Song A, White B, Winbush T, Aceves C M, Anderson C, Gangavarapu K, Hufbauer E, Kurzban E, Lee J, Matteson N L, Parker E, Perkins S A, Ramesh K S, Robles-Sikisaka R, Schwab M A, Spencer E, Wohl S, Nicholson L, Mchardy I H, Dimmock D P, Hobbs C A, Bakhtar O, Harding A, Mendoza A, Bolze A, Becker D, Cirulli E T, Isaksson M, Schiabor Barrett K M, Washington N L, Malone J D, Schafer A M, Gurfield N, Stous S, Fielding-Miller R, Garfein R S, Gaines T, Anderson C, Martin N K, Schooley R, Austin B, Maccannell D R, Kingsmore S F, Lee W, Shah S, Mcdonald E, Yu A T, Zeller M, Fisch K M, Longhurst C, Maysent P, Pride D, Khosla P K, Laurent L C, Yeo G W, Andersen K G, Knight R. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. ***Nature*** **609;2022:101-108.**

This study describes methodology for wastewater sequencing of SARS-CoV-2 RNA, and demonstrates the efficacy of the methodology for tracking the evolution of infection in a circumscribed geographic region.

Analysis of viral genomic sequences in wastewater promises to circumvent many epidemiologic problems associated with reliance on clinical samples, including biased sampling and high cost. However, acquisition of high-quality sequence information from wastewater is technically challenging because, in wastewater, the viral RNA is heavily fragmented, and low in concentration. Additionally, wastewater contains PCR inhibitors. The authors concentrate viral RNA using affinity capture magnetic hydrogel particles and screen RNA eluted from them for SARS-Cov-2 RNA by real-time RT-qPCR using 3 gene targets (N1, N2 and E-gene). They sequence positive samples using tiled amplicons made with a primer set from Swift Biosciences. They employ a protocol especially miniaturized for the purpose, and achieve nearly complete viral genome coverage. Additionally, they develop a tool to estimate the relative abundance of different viral lineages in mixed samples. With these methods, the authors conduct longitudinal viral surveillance over a 10-month period at the campus of the University of California at San Diego, collecting daily samples from 131 wastewater samplers covering 360 campus buildings (19,944 samples in total). Notably, they detect emerging variants of concern up to 2 weeks earlier than surveillance with clinical samples, and avoid the serious biases to which clinical sampling is susceptible. The methodology is offered as a viable option for SARS-CoV-2 monitoring in communities.

MASS SPECTROMETRY

Harrison J A, Pruška A, Bittner P, Muck A, Cooper-Shepherd D A, Zenobi R. Advancing cyclic ion mobility mass spectrometry methods for studying biomolecules: toward the conformational dynamics of mega dalton protein aggregates. *Analytical Chemistry* 94;2022:12435-12443.

This study provides information useful in selecting instrument parameters for the analysis of high mass protein and oligonucleotide ions by ion mobility spectroscopy using the recently released cyclic traveling wave platform from Waters Corp., Milford MA. In this platform, the ion mobility separator is positioned orthogonally to the main ion optical axis, and ion guides on either side of it are used for injection, ejection, storage, and activation of ions. An array of multiple electrodes generates the traveling wave, and ions can be moved between the ion mobility separator and the pre- and post-separator ion guides by changing the direction of the wave on the array. A quadrupole mass filter pre-selects ions for analysis, and ions are detected downstream with a time-of-flight analyzer. The authors show how the effects of the voltages applied to the various components of the system, and the interplay between these voltages, affect ion transmission and arrival time distribution for ions of *m/z* 2000-10,000. They also indicate how instrument parameters must be optimized for ions of different *m/z*. The authors also illustrate effects of varying the temperature in a temperature-controlled nanoelectrospray source.

James V K, Sanders J D, Aizikov K, Fort K L, Grinfeld D, Makarov A, Brodbelt J S. Advancing Orbitrap measurements of collision cross sections to multiple species for broad applications. *Analytical Chemistry* 94;2022:15613-15620.

The collisional cross-section of gas-phase ions is generally measured in dedicated ion mobility spectrometers, but it may also be derived from the rates of signal decay in ion trapping analyzers, either in the time or in the frequency domain, although with poorer precision and possibly poorer accuracy due to collisional fragmentation at the higher kinetic energies of ions in Orbitrap or Fourier transform-ion cyclotron resonance systems. The present paper describes computational methods, based on direct decay profile fitting, that permit estimation of collisional cross-section from commonly available mass spectra acquired in an Orbitrap mass spectrometer, avoiding the need for access to transient or complex Fourier spectra, or isolation of individual charge states of ions prior to cross-section analysis. Indeed, the authors determine collision cross-section values for multiple ions of different *m/z* simultaneously. This capability is useful when specific ions or charge states cannot be isolated for independent study, and reduces data acquisition time when monitoring collision cross-section of multiple charge states of proteins. The new methodology is hoped to broaden access to collision cross-section measurement for diverse studies, including the monitoring of collision-induced unfolding of native-like proteins.

Knizner K T, Guymon J P, Garrard K P, Bouvrée G, Manni J, Hauschild J-P, Strupat K, Fort K L, Earley L, Wouters E R, Pu F, Radosevich A J, Elsen N L, Williams J D, Pankow M R, Muddiman D C. Next-generation infrared matrix-assisted laser desorption electrospray ionization source for mass spectrometry imaging and high-throughput screening. *Journal of the American Society for Mass Spectrometry* 33;2022:2070-2077.

Matrix-assisted laser desorption electrospray ionization (MALDESI) is a hybrid, ambient ionization method in which a laser desorbs neutrals from a solid sample, then the neutrals partition into charged droplets from an orthogonal electrospray plume and become ionized in an electrospray-like manner prior to mass spectral analysis. Infrared (IR) lasers work in MALDESI by resonantly exciting O–H stretching of water that is either endogenous to the sample or supplied exogenously as an applied layer of ice, thereby avoiding the need for the organic matrix that is applied in classical matrix-assisted laser desorption/ionization (MALDI). IR-MALDESI has been used in mass spectral imaging and high-throughput screening for drug discovery. Here, an improved IR-MALDESI source design is described. It incorporates a vertically mounted IR-laser, a computer-controlled sample stage, an aluminum enclosure, and a novel mass spectrometer interface plate for coupling to Orbitrap mass spectrometers. The authors document the ease of use and versatility of the new design and show improvements in ion abundance over a wide range of *m/z* values.

MACROMOLECULAR SYNTHESIS & SYNTHETIC BIOLOGY

Dauparas J, Anishchenko I, Bennett N, Bai H, Ragotte R J, Milles L F, Wicky B I M, Courbet A, De Haas R J, Bethel N, Leung P J Y, Huddy T F, Pellock S, Tischer D, Chan F, Koepnick B, Nguyen H, Kang A, Sankaran B, Bera A K, King N P, Baker D. Robust deep learning-based protein sequence design using ProteinMPNN. *Science* 378;2022:49-56.

Given a protein backbone structure of interest, Dauparas *et al.* tackle the problem of how best to design an amino acid sequence that will fold into this structure. Physical approaches to this design problem, such as Rosetta, identify the lowest energy sequence for the desired structure, and then, in a second step, perform structure prediction to ensure that even lower energy structures aren't available to that sequence and that the sequence won't aggregate or form multimers, avoidance of which would require further sequence customization. Instead, the authors here propose a deep-learning sequence design method, Protein MPNN, based on a message-passing neural network architecture trained to find the most probable amino acids for the protein backbone given all the examples in the protein database. For native protein backbones, ProteinMPNN has a sequence recovery of 52.4%, compared to 32.9% for Rosetta. ProteinMPNN requires much less time, and rescues designs that Rosetta or AlphaFold previously failed to achieve. The work improves upon previous implementations of message-passing neural network architecture by extending design from monomers to cyclic oligomers, protein nanoparticles and protein-protein interfaces, and by extensive validation of structures by crystallography and cryo-electron microscopy. The authors also note that ProteinMPNN generates sequences that are predicted to fold more confidently and accurately than the original native sequences, suggesting that they might be useful for improving expression and stability of recombinantly expressed native proteins.

GLYCANS

Li Q, Levi S M, Wagen C C, Wendlandt A E, Jacobsen E N. Site-selective, stereocontrolled glycosylation of minimally protected sugars. *Nature* 608;2022:74-79.

Control of reaction site-specificity during non-enzymatic synthesis of oligosaccharides is traditionally accomplished with the use of protecting groups. But the need to install and, later, to remove protecting groups adds to the complexity of synthesis, and may result in steric or electronic deactivation of the unprotected hydroxyls that one wishes to react. Li *et al.* here contribute to investigation of alternative, catalyst-controlled approaches for performing site-selective glycosylation reactions. Their approach exploits attractive, non-covalent interactions between carbohydrates and electron-rich aromatic side chains known to occur in interactions between carbohydrates and proteins. Such attraction is based on C–H/π interactions. The authors design bis-thioureas bearing electron-rich arenes to act as small-molecule catalysts for promotion of highly stereo- and site-specific glycosylations of unprotected or minimally protected mono- and di-saccharides. They accomplish highly (1,2)-selective galactosylations and mannosylations of β-carbohydrates in this way. The results suggest that stabilizing non-covalent interactions may be developed into a general approach to selective functionalization of carbohydrates in glycan synthesis.

Yao W, Xiong D-C, Yang Y, Geng C, Cong Z, Li F, Li B-F, Qin X, Wang L-N, Xue W-Y, Yu N, Zhang H, Wu X, Liu M, Ye X-S. Automated solution-phase multiplicative synthesis of complex glycans up to a 1,080-mer. *Nature Synthesis* 1;2022:854-863.

Yao *et al.* report the design and application of an automated synthesizer for solution-phase assembly of glycans of extended length. Glycosyl donors are pre-activated (in the absence of glycosyl acceptor) by a promoter - either trifluoromethanesulfonic anhydride and diphenyl sulfoxide or *p*-toluenesulfenyl chloride and silver triflate. Alternatively, glycosyl donors are photoactivated with the copper(II) salt of trifluoromethanesulfonic acid (Umemoto's reagent) and ultraviolet irradiation. The authors assemble a library of oligosaccharides synthesized from protected monosaccharide building blocks, then assemble longer glycans by linking oligosaccharides together. Among the glycans synthesized are the Fontaparinux pentasaccharide, an anti-thrombotic agent, and a 1,080-mer arabinan, a polymer of arabinose.

PROTEOMICS

Naylor B C, Anderson C N K, Hadfield M, Parkinson D H, Ahlstrom A, Hannemann A, Quilling C R, Cutler K J, Denton R L, Adamson R, Angel T E, Burlett R S, Hafen P S, Dallon J C, Transtrum M K, Hyldahl R D, Price J C. Utilizing nonequilibrium isotope enrichments to dramatically increase turnover measurement ranges in single biopsy samples from humans. *Journal of Proteome Research* 21;2022:2703-2714.

Understanding the control of protein abundance requires knowledge of protein turnover rates. *In vivo* turnover rates may be measured most conveniently by supplying deuterium in the form of deuterated water (^2H_2O), which is incorporated first into amino acids and hence into proteins. The abundance of newly synthesized proteins may then be quantified by mass spectrometry to calculate turnover rates. Classically, the amount of deuterium in the experimental subject is increased as rapidly as possible. The abundance of deuterium in proteins then rises to a maximum, whereupon equilibrium is reached. The rate of rise is determined by each protein's turnover rate. Unfortunately, turnover rates span a very large range - 4 orders of magnitude. Given the impracticability of taking biopsies at multiple time points to accommodate this huge range, the time at which any one biopsy is taken curtails the span of turnover rates that yield signal-to-noise ratios that can be satisfactorily measured. To alleviate this limitation, Naylor *et al.* apply a constantly increasing quantity of deuterium rather than a step increase. Under this condition, even proteins with rapid turnover don't reach deuterium saturation (equilibrium). Signal-to-noise ratios reach a maximum for each protein at the protein's half-life, and stay there. This enables measurement of turnover rates over a much bigger span of values with a single biopsy. Using their present protocol for non-equilibrium enrichment, the authors extend the range of measurable turnover rates from ~10-fold to ~4000-fold in a single muscle biopsy sample. They provide an open-source data analysis tool, DeuteRater-H, for calculation of turnover rates in such experiments.

FUNCTIONAL GENOMICS/PROTEOMICS

Bhattarai-Kline S, Lear S K, Fishman C B, Lopez S C, Lockshin E R, Schubert M G, Nivala J, Church G M, Shipman S L. Recording gene expression order in DNA by CRISPR addition of retron barcodes. *Nature* 608;2022:217-225.

This study introduces a new analog molecular recorder system for making a time-ordered record of transcriptional events in a living genome. The system makes use of CRISPR-Cas integrases (Cas1 and Cas2) to insert specific DNA sequences into a bacterial cell's CRISPR array. The authors add to this the use of retrons – small genomic DNA sequences from prokaryotes that can be customized to synthesize compact, specific DNA molecular tags *in vivo*. Retrons with different sequence, placed under the control of different promoters of interest in a single cell, transcribe their tag sequence into RNA when their promoter is activated. The RNA is reverse transcribed into DNA by the retron reverse transcriptase to act as a 'receipt' for transcription from the promoter. The DNA receipt is then bound by Cas1-Cas2, and integrated into the cell's CRISPR array, thus creating a permanent record of the transcription. When another tagged promoter is activated, a different receipt is generated and gets integrated into the CRISPR array in the position following the first spacer. The overall result is a linear record of transcription receipts in individual bacterial cells that can be used to reconstruct the temporal history of transcriptional events experienced by that cell. The number of distinct signals is limited only by the number of different barcodes in the library of retrons employed. The system is anticipated to be most immediately useful for recording the presence of environmental pollutants, metabolites and pathogens.

Choi J, Chen W, Minkina A, Chardon F M, Suiter C C, Regalado S G, Domcke S, Hamazaki N, Lee C, Martin B, Daza R M, Shendure J. A time-resolved, multi-symbol molecular recorder via sequential genome editing. *Nature* 608;2022:98-107.

Choi *et al.* explore a molecular recorder system with characteristics similar in many ways to the system of Bhattarai-Kline *et al*. described above. But it does not use a CRISPR array or retrons, so it requires no adaptation for use in mammalian cells. Choi *et al.* begin with a tandem array of partial CRISPR-Cas9 target sites. All but the first site are truncated at their 5′ ends, so they are inactive. A cellular event triggers expression of a prime editing guide RNA, together with the prime editing enzyme. These molecules mediate insertion of a short DNA barcode into the sole active site in the tandem array (the 5′-most target site), plus a 3 bp key that activates the next target site. In this way, events are recorded sequentially. This system allows the authors to record complex event histories in HEK293T cells. In conjunction with single-cell RNA sequencing, the authors further reconstruct a monophyletic lineage of 3,257 cells accumulating sequential insertions across at least 20 generations and 25 days of *in vitro* clonal expansion.

Deng Y, Bartosovic M, Ma S, Zhang D, Kukanja P, Xiao Y, Su G, Liu Y, Qin X, Rosoklija G B, Dwork A J, Mann J J, Xu M L, Halene S, Craft J E, Leong K W, Boldrini M, Castelo-Branco G, Fan R. Spatial profiling of chromatin accessibility in mouse and human tissues. *Nature* 609;2022:375-383.

A single-cell implementation of assay for transposase-accessible chromatin using sequencing (ATAC-seq) is here developed for spatially resolved profiling of cells in tissue sections in order to capture spatial epigenetic information. Tn5 transposition is performed on tissue sections lightly fixed with formaldehyde. Two sets of DNA barcodes are then added to the genomic DNA oligonucleotides formed at Tn5-accessible sites. First, DNA barcode A solutions are delivered by microchannel-guided flow to perform *in situ* ligation reactions for

appending distinct spatial barcodes, Ai (where i = 1-50). Then, a second set of barcodes, Bj (where j = 1-50), are introduced using another set of microchannels orthogonal to the first set of channels. Ligation of the barcodes at the intersection points of the two sets of channels thus defines a set of 2,500 tissue pixels containing distinct combinations of barcodes Ai and Bj. Library construction is then completed during PCR amplification for sequencing. The authors use this scheme to map the accessible genome during embryonic development of the mouse nervous system. They map the mouse and human brain to reveal spatially distinct epigenetic signatures. They also distinguish different types of immune cells in follicular and extrafollicular zones of human tonsils.

IMAGING

Chen B, Chang B-J, Roudot P, Zhou F, Sapoznik E, Marlar-Pavey M, Hayes J B, Brown P T, Zeng C-W, Lambert T, Friedman J R, Zhang C-L, Burnette D T, Shepherd D P, Dean K M, Fiolka R P. Resolution doubling in light-sheet microscopy via oblique plane structured illumination. ***Nature Methods*** **19;2022:1419-1426.**

The authors of this paper describe a new, convenient way to combine light sheet fluorescence microscopy (LSFM), a technique that provides volumetric imaging while minimizing phototoxicity, and structured illumination microscopy (SIM), a technique that provides high resolution exceeding the diffraction limit. Importantly, they accomplish this goal while avoiding the need for multiple illumination objectives. Instead, they illuminate the sample with a structured light sheet emitted at an angle from a single objective that can be rotated to produce the multidirectional illumination needed for SIM. This arrangement doubles (to ∼150 nm) the resolution achieved by LSFM in the lateral direction with the use of structured illumination, while retaining low rates of photobleaching. The authors add a rapid image rotator to minimize acquisition time. They achieve volumetric acquisition speeds exceeding 1 Hz – faster than SIM instruments have hitherto achieved when imaging in three dimensions. The image rotator steers the illumination with two galvanometric mirrors and returns fluorescence light *via* one of three angled static mirrors to achieve optimal rotation of the structured light sheets and returning fluorescence images.

Toi P T, Jang H J, Min K, Kim S-P, Lee S-K, Lee J, Kwag J, Park J-Y. In vivo direct imaging of neuronal activity at high temporospatial resolution. ***Science*** **378;2022:160-168.**

Functional magnetic resonance imaging (fMRI) measures changes in blood oxygenation-dependent (BOLD) signal when neuronal activity changes. The hemodynamic response measured by fMRI is a surrogate for brain electrical activity. Its spatial precision is limited by the vascular architecture. Its temporal precision is limited by the lag in hemodynamic response to neural activity. Toi *et al*. propose a new approach to fMRI that enables direct response to neuronal activity in the millisecond range, a timescale more closely commensurate with neuronal activity responses, while retaining the inherently high spatial resolution of fMRI (0.22 mm). They implement a two-dimensional (2D) fast line-scan acquisition strategy that does not require hardware changes to

a 9.4 tesla scanner that they employ. In studies of the neural response to stimulation of the whiskers of anaesthetized mice, the methodology detects temporal delays in electrical activity between corticothalamic layers. The results therefore suggest that the new methodology will provide enhanced capabilities for investigation of the spatiotemporal dynamics of neural networks.

Yoon H H, Fernandez H A, Nigmatulin F, Cai W, Yang Z, Cui H, Ahmed F, Cui X, Uddin M G, Minot E D, Lipsanen H, Kim K, Hakonen P, Hasan T, Sun Z. Miniaturized spectrometers with a tunable van der Waals junction. *Science* 378;2022:296-299.

The miniaturization of spectrometers is desirable for applications that require portability or biological implantability, but miniaturization has traditionally incurred diminished performance in spectral resolution and light sensitivity. Yoon *et al.* now describe a spectrometer with the remarkable footprint dimensions of 22 μm x 8 μm. It displays spectral resolution approaching benchtop systems (~3 nm), high peak wavelength accuracy (~0.36 nm), and broad operating bandwidth (~405-845 nm). Whereas benchtop spectrometers utilize a dispersive element such as a prism or diffraction grating in conjunction with an array of detectors, the new device utilizes mathematical algorithms for spectrum reconstruction from signals collected by a single detector. The detector is formed by a two-dimensional (2D) semiconductor comprising an overlapping heterojunction of two 2D semiconducting materials, molybdenum disulfide (MoS_2) and tungsten diselanide (WSe_2). The junction is electrically tunable – the current generated by the semiconductor in response to photons at different applied voltages is used to reconstruct the spectrum of incident light. The spectral resolution of the device is much better than existing reconstructive devices. Such a spectrometer promises unprecedented capability to investigate spectral properties of biological systems in the visible range.

CELL BIOLOGY

Amadei G, Handford C E, Qiu C, De Jonghe J, Greenfeld H, Tran M, Martin B K, Chen D-Y, Aguilera-Castrejon A, Hanna J H, Elowitz M B, Hollfelder F, Shendure J, Glover D M, Zernicka-Goetz M. Embryo model completes gastrulation to neurulation and organogenesis. *Nature* 610;2022:143-153.

Tarazi S, Aguilera-Castrejon A, Joubran C, Ghanem N, Ashouokhi S, Roncato F, Wildschutz E, Haddad M, Oldak B, Gomez-Cesar E, Livnat N, Viukov S, Lokshtanov D, Naveh-Tassa S, Rose M, Hanna S, Raanan C, Brenner O, Kedmi M, Keren-Shaul H, Lapidot T, Maza I, Novershtern N, Hanna J H. Post-gastrulation synthetic embryos generated ex utero from mouse naive ESCs. *Cell* 185;2022:3290-3306.e3225.

During embryonic development, the zygote divides to form a ball of cells termed the blastocyst that will implant into the uterine wall. The blastocyst contains epiblast cells that will form the organism, primitive endoderm cells that will contribute to the yolk sac, and trophectoderm cells that will contribute to the placenta. Embryonic stem (ES) cells, which are derived from the epiblast, can be induced to form many embryo-like

structures *ex utero*, but they don't accurately undergo gastrulation movements and don't recapitulate development to neurulation. Two groups now overcome these hurdles by co-aggregating mouse ES cells *in vitro* with cells that emulate the other blastocyst cell types. Both groups add a mouse ES cell line that transiently overexpresses the master regulator GATA4, which is also expressed by the primitive endoderm. Amadei *et al.* add to the mix a trophoblast stem cell line. Tarazi *et al.* add ES cells transiently expressing Cdx2, which primes them toward the trophectoderm lineage. With optimization of culture conditions, both groups report that the resulting 'embryoids' develop further than has hitherto been achieved *in vitro*, displaying structures similar to 8.5-day mouse embryos (E8.5 stage), including forebrain and midbrain regions, a beating heart-like structure, a trunk with a neural tube and somites, a gut tube, and primordial germ cells. This major advance foreshadows the development of experimental models for investigation of advanced post-implantation development.

DRUG DESIGN & DEVELOPMENT

Agarwalla P, Ogunnaike E A, Ahn S, Froehlich K A, Jansson A, Ligler F S, Dotti G, Brudno Y. Bioinstructive implantable scaffolds for rapid in vivo manufacture and release of CAR-T cells. *Nature Biotechnology* 40;2022:1250-1258.

T cells expressing chimeric antigen receptors (CAR-T cells), engineered for specificity against target cells of interest, have generated much enthusiasm for their use in the treatment of malignancies and other clinical conditions. But widespread use of this therapeutic modality has been impeded by the lengthy, complex, and expensive procedures for generating CAR-T cells ready for administration to patients. Agarwalla *et al.* here describe a process that streamlines CAR-T cell manufacturing and promises to reduce processing time to just a single day. The core of the process is the use of an alginate scaffold, implantable within the patient, that can host T cells and transducing viral particles, stimulate T cell activation and proliferation, promote T cell transduction, locally expand CAR-T cells, and sustainably release the functional CAR-T cells to control tumor growth. The alginate scaffold uses FDA-approved, non-immunogenic materials. It is seeded with patient's mononuclear cells and retrovial particles encoding a CAR that is specific for the target antigen. This arrangement functions to limit off-target gene transfer. Anti-CD3 and anti-CD28 antibodies immobilized on the scaffold mediate cell activation and interleukin-mediated proliferation. After subcutaneous implantation of the scaffold, fully functional, reprogrammed CAR-T cells are released. The methodology is successfully tested in a mouse xenograft model of lymphoma. It is hoped to provide a platform for more general reprogramming and release of therapeutic cells going forward.

Foy S P, Jacoby K, Bota D A, Hunter T, Pan Z, Stawiski E, Ma Y, Lu W, Peng S, Wang C L, Yuen B, Dalmas O, Heeringa K, Sennino B, Conroy A, Bethune M T, Mende I, White W, Kukreja M, Gunturu S, Humphrey E, Hussaini A, An D, Litterman A J, Quach B B, Ng A H C, Lu Y, Smith C, Campbell K M, Anaya D, Skrdlant L, Huang E Y-H, Mendoza V, Mathur J, Dengler L, Purandare B, Moot R, Yi M C, Funke R, Sibley A, Stallings-Schmitt T, Oh D Y, Chmielowski B, Abedi M, Yuan Y, Sosman J A, Lee S M, Schoenfeld A J, Baltimore D, Heath J R, Franzusoff A, Ribas A, Rao A V, Mandl S J. Non-viral

precision T cell receptor replacement for personalized cell therapy. *Nature* 2022: Nov 10. doi: 10.1038/s41586-022-05531-1 (Online ahead of print)

In the setting of a Phase 1 clinical trial, this study demonstrates the manufacture and delivery of tumor-specific CAR-T cells using a non-viral approach based on CRISPR-Cas9 precision gene editing. This approach is used on the patient's own circulating T cells to simultaneously knock-out the endogenous genes encoding TCRα and TCRβ, and to knock-in to the locus genes for the two chains of a CAR that is specific for tumor neoantigens. Sixteen patients with refractory solid tumors (including breast and colon) are included in the clinical trial. Each patient receives CAR T cells specific for 3 distinct neoantigens. The study successfully demonstrates feasibility and safety. The authors anticipate future exploitation of the versatility of CRISPR-Cas9 gene editing in this context to incorporate additional gene edits to improve T cell function by, or example, abrogating T cell exhaustion and avoiding immune suppression in the solid tumor environment. They hope also to accomplish *in vivo* T cell expansion without the present need for lymphodepleting conditioning chemotherapy.